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博士（スポーツ科学）

Relationship between body composition and resting energy expenditure of male athletes

男性アスリートの身体組成と安静時代謝量の関係

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Chapter 1

Introduction

1) Importance of resting energy expenditure for athletes

Athletes strive to achieve an optimum sport specific body size, body composition, and energy stores to maximize their performance. To pursue these objectives, athletes need to have good nutritional practice to manage fat, protein and carbohydrate balances separately (1). However, part of the nutritional challenge for an athlete is that the body possesses no mechanism for automatically accommodating energy intake either to the oxidation of specific metabolic fuels or to the expenditure of energy in general by working muscle (2). Hence, many of athletes become energetically insufficient. When energy intake is insufficient, athletes cannot perform their best performance and both body fat and body protein will be used for energy source. However, the more protein is used for energy, it increases the breakdown of body protein from fluid and tissue including skeletal muscle, and ultimately tissue repair slows and muscle size and strength diminish, resulting in decreased physical performance (3). Additionally, if energy intake is limited, the opportunity to obtain other essential nutrients necessary for optimal sport performance and good health will be restricted.

On the other hand, if the excess calories were consumed above the day's nutritional requirement, nearly all the excess of calories will be stored in the body as fat. Accumulation of excess body fat become burden on the joints and skeletal framework, as a consequence it may lead to injuries significantly influence athletic life. Moreover, some athletes required to increase their body weight through diet and weight training, with the goal of becoming larger than their opponents such as American football linemen (4). Body weight gain is ideally accomplished by increasing muscle mass, but often players increase their body size by accumulating more adipose tissue, especially in abdominal region. As a result, there have been many studies reported the high prevalence of metabolic syndrome and

cardiovascular risk for American football players (5,6).

Competitive athletes commonly aim to modify their bodies to achieve their goal by reducing body fat while increasing fat-free mass (FFM). To guide the progress of athletes, they need to eat by discipline based on their individual nutritional needs within the carefully monitored energy balance. Energy balance can be defined as follows; energy balance = energy intake – energy expenditure. This is the first law of thermodynamics introduced by Clausius and in order for athletes as well as other support stuff such as dietitian to utilize the idea, the quantitative information regarding energy intake and expenditure become important.

Resting energy expenditure (REE) is often used as a basis for estimating an individual's total energy expenditure, and therefore also basis for energy needs as well. Total daily energy requirement can be predicted by multiplying REE by physical activity level (PAL), as noted in the report from World Health Organization and *Dietary Reference Intakes for Japanese*, 2010 (DRIs) (7,8) (Table 1-1). Therefore, in order to accurately predict energy expenditure to determine the individual nutritional requirement and manage the energy balance, a reliable accurate and practical way to estimate REE for athlete is crucial. In this thesis, REE for male athletes in relationship with their body composition contributing to accuire more evidence for better estimation of REE is the main emphasis.

2) Definition of resting energy expenditure

Basal metabolic rate and resting energy expenditure are typically used as synonyms. However, they actually have fixed definition as follows; Basal metabolic rate (BMR) is defined as the minimal amount of energy expended that is compatible with life. Measuring oxygen consumption under stringent conditions indirectly determines the BMR (9,10). It reflects the amount of energy used over 24

hours while physically lying down and mentally resting in a thermoneutral environment that prevents the activation of heat-generating processes such as shivering. Moreover, BMR measurements are made early in the morning, before the person has engaged in any physical activity to reduce other calorigenic influence. Subjects also has to be fasted (having eaten no food except for water) for at least 12 hours prior to a test to avoid increases in metabolism from digestion, absorption, and assimilation of ingested nutrients. After subject rest supine for about 30 minutes in a comfortable environment, then oxygen consumption is measured for 10 minutes twice consecutively (11). If any of the conditions for BMR are not met, energy expenditure at rest should be referred to as resting energy expenditure (REE). BMR is now rarely measured for practical reasons, since measuring REE does not require strict condition as it does with BMR.

Although, there are different definition for BMR and REE, the difference between the two measurements is said to be within 10%, which is same as the coefficient of variation measured in different day (11). In that reason, "REE" was used as generic term for both "BMR" and "REE" in the current text, unless references articles or figures used a certain defined term. In our research studies introduced in the thesis also uses "REE" according to the definition, even though the measurements were practiced under most of the conditions required for BMR measurement.

3) Progress of resting energy expenditure estimation

Calorimetry has provided important insights regarding the energy metabolism in humans and other animals over the last century (12). Since then, people started to understand how energy metabolism responds to exercise, food intake, disease, and the environment. One of the most important motivations for the development in the field of energy metabolism studies were to establish standard values for the

various components of energy expenditure to be able to provide adequate recommendations of energy intake in various circumstances. The emphasis has been especially given to develop standards of REE because it is relatively easy to measure compared to measuring energy expenditure during physical activities. It is also usually the largest component of total energy expenditure (Figure 1-1). In fact, REE accounts for about 60-70% of normal adult.

The basis for using different parameter to predict metabolic rate was first made emphasis on surface area in regards to the influence of heat loss, heat production, sites of heat production, and mechanisms of heat production. Rubner in 1883 (13) proposed the "surface law" by using his dogs and found that the metabolic rate of different dogs elevated in relation to their surface area. Later clinicians from Mayo Clinic published the study with large number of subjects presenting the reference values for metabolic rate based on individual surface area (14). However, the relationship between metabolic rate and surface area was so variable. Additionally, there were some discrepancies in surface law between male and females or between different age groups (15,16).

Surface area was typically estimated based on body height and weight. At the same time, it was one of the most difficult anthropometric characteristics to measure. Therefore, it is reasonable on theory to use physical characteristics such as weight or height that can be easily and accurately measured than surface area to estimate REE. Harris and Benedict (17) published in 1919 their prediction equations in relation to weight and height which have been widely used to the present day. Since then there have been many other equations were published for estimating REE based on age, sex, surface area, weight and height.

In Japan, Ministry of Health, Labour and Welfare has published the reference values for metabolic rate per body weight which were categorized by sex and age

group in the Dietary Reference Intakes for Japanese, 2010 (DRIs) (8). The reference values were established based on the multiple studies with different groups of Japanese subjects followed the typical protocol of the REE measurement (8). Therefore, body weight with their specific age group and sex are used to estimate REE for typical healthy Japanese.

4) Body composition and resting energy expenditure

Meanwhile, some group of researchers argued that by studying the relationship of REE with the size of tissues and organs which are the source of heat may provide a rational explanation for the irregularities in the surface law (18). Body composition can explain some of the irregularity of REE which could not explained by the surface law. For example, lower metabolic rate per body weight (kg) in women compared to men is due to the presence of greater amount of adipose tissue.

Adipose tissue is specialized connective tissue that functions as the major storage site for body fat in the form of triglycerides. It is known to have a low O_2 consumption, which means low metabolic rate, compared to other tissues. However, even though the metabolic rate of adipose tissue is low, it may add to the variance in REE, especially for overweight and obese individuals. They were reported to have a higher REE than their lean counterpart, even after adjusted by FFM (19). For overweight and obese people, if REE was adjusted by fat mass then most of the between- group differences disappeared.

On the other hand, FFM is the major determinant of REE especially for non-obese subjects and explains about 60-80% of its variance based on the studies with wide weight range (12,20,21). Therefore, it was suggested that it may be more useful to utilize metabolic rate in relation to FFM for estimating REE than utilizing body weight, height or surface area. FFM can be divided into different

functional body components such as metabolically active tissues and very low metabolically active tissues. For example, liver, brain, heart, and kidney have metabolic rates of 200, 240, 440, and 440 kcal/kg/day, respectively (12) (Table 1-2), as compared to bone, which is quite low at 2.3 kcal/kg/day. Even skeletal muscle, which is commonly considered a metabolically active tissue, has a metabolic rate at only 13 kcal/kg/day. As a consequence, if proportional contribution of different organs and tissue mass to FFM changes, it changes REE/FFM ratio which have been observed in the previous studies.

5) Issues on resting energy expenditure estimation for male athletes

For the case of athletes, they typically have more FFM than a non-athletic counterpart with similar body weight. Therefore, FFM was believed to be more suitable to estimate REE rather than utilizing their body weight which was commonly apply for general non-athletic population. Previous study has reported that there was no difference in metabolic rates per FFM between the groups with rowers, runners, and non-athletic controls (22). It was suggested that metabolic rate of FFM is not influenced by habitual types of exercise or frequency to participate in exercise per se. Therefore, Japan Institute of Sports Science (JISS) has decided to utilize FFM for REE estimation specific for athletes as follows; REE for athletes (kcal/day) = 28.5(kcal/kg/day)×FFM(kg) (23) . However, the REE/FFM ratio (28.5kcal/kg/day) in the formula is based on the previous studies with untrained population, thus the formula is not established based on the actual REE measurements of athletes. Moreover, there has been many studies found that the REE of FFM (REE/FFM ratio) decreases as FFM becomes large (12,24,25). If this is true, there will be a trend of over-estimation or under-estimation depending on a size of FFM since athletes have wide range of FFM. Therefore, for these two

reasons, the adequacy of using the formula utilizing FFM needs to be investigated by collecting the actual REE and body composition measurements of athletes.

While the relationship between FFM and REE has been previously explored at organs and tissue level, (26-29), such studies were largely done on healthy but untrained individuals. However, it is not known that the same findings would apply to athletic population. Athletes typically train daily, and have a body composition which is different from the norm and is characterized by a large FFM (30). Therefore, there may be different, relatively unique factors for athletes that might influence the value of REE. In fact, Grund et al. (31) found that resistance trained men had higher REE compared to untrained men even after REE was evaluated as a ratio divided by FFM. However, the explanation for this finding has yet to be explained.

6) Purpose of the thesis

Good nutritional practice will made possible for athletes to train hard, recover quickly and adapt more effectively with less risk of illness and injury. Accurately assessing individual total energy expenditure is the foundation of good nutritional practice and the management the energy balance. Since REE is the basis for predicting total energy expenditure, accurately estimating REE is a great matter for athletes.

The factors influences a inter-individual variation in REE related to body size were considered to be mainly as follows; 1) FFM;, 2) proportional contribution of different organ mass (for example, skeletal muscle, masses of the brain, liver, heart, and kidneys) to FFM; 3) variations in organ metabolic rates; and 4) adaptations of specific metabolic rates in response to both overfeeding and underfeeding (adaptive thermogenesis) (32). In this thesis, it will be mainly focus on the body composition

and its relation to REE. Even though there were multiple studies investigated the relationship between body composition and REE, it is not clearly understood if the same findings would apply to athletic population. There may be different, relatively unique factors for athletes that might influence the value of REE which is not seen in non-athletic subjects.

Therefore, the purpose of the thesis was to investigate the relationship between body compositions and REE of male athletes with the emphasis on FFM components, including internal organs mass, and the influence of relative contributions of FFM components on REE. The findings of the studies would provide scientific evidence which contribute for better estimation of REE specific towards athletic population.

7) Composition of the studies

Study 1 (Chapter 2): Evaluate the relationship between FFM and REE at the organ-tissue level for male athletes using a four organ-tissue compartment.

Study 2 (Chapter 3): Investigate the influence of FFM gain on REE of male power athletes through one year in longitudinal study.

Study 3 (Chapter 4): Examine the influence of internal organs to REE/FFM ratio in male power athletes.

Table 1-1

Classification of lifestyles in relation to the intensity of habitual physical activity, or PAL

Category	PAL value
Sedentary or light activity lifestyle	1.40-1.69
Active or moderately active lifestyle	1.70-1.99
Vigorous or vigorously active lifestyle	2.00-2.40*

(Report from WHO,1985)

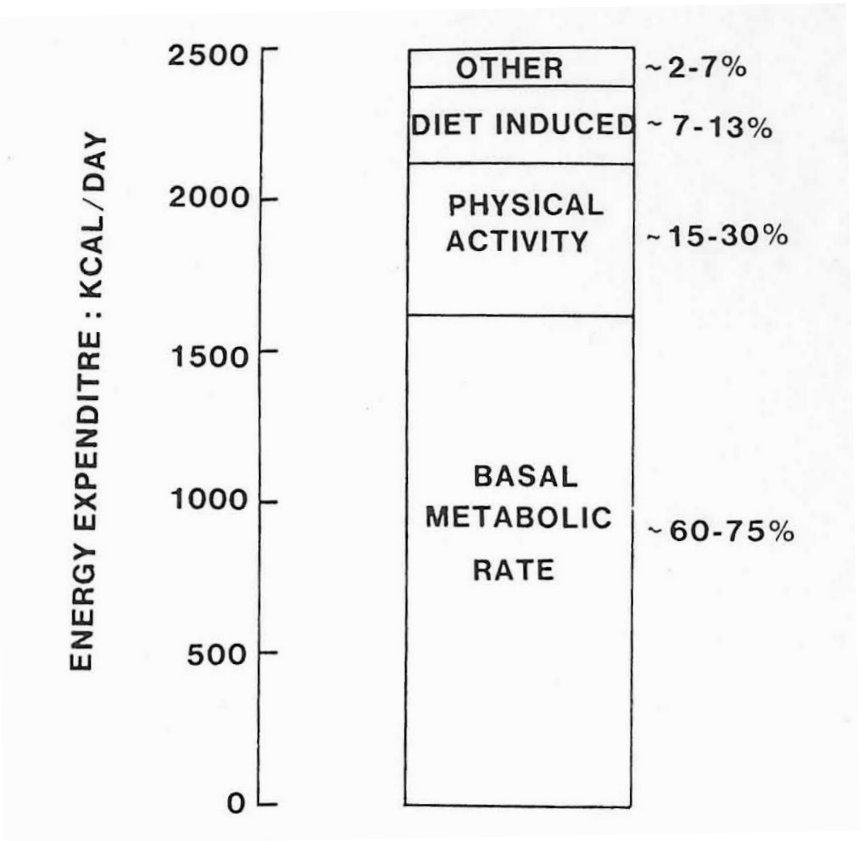

Figure 1-1 Components of total energy expenditure (Elia M.,1992)

Table 1-2

Contribution of different organs and tissues to body weight and resting metabolic rate

	Tissue or organ weight (kg)	Tissue or organ weight (% body weight)	Organ metabolic rate (kcal/kg/day)	Metabolic rate (% total)
Liver	1.8	2.57	200	21
Brain	1.4	2	240	22
Heart	0.33	0.47	440	9
Kidneys	0.31	0.44	440	8
Muscle	28	40	13	22
Adipose tissue	15	21.43	4.5	4
Miscellaneous (tissues by difference such as bone, skin, intestines, glands)	23.16	33.09	12	16
Total	70	100	100(1680kcal/day)	

(Elia M.,1992)

Chapter 2

Relationship of fat-free mass and resting energy expenditure of male athletes

(J Nutr Scie Vitaminol, 57, 394-400, 2011)

INTRODUCTION

Resting energy expenditure (REE) is often used as a basis for estimating an individual's energy requirement (EER). EER can be roughly assessed by multiplying REE by physical activity level (PAL), as noted in *Dietary Reference Intakes for Japanese*, 2010 (DRIs) (8). Therefore, an accurate way to estimate and predict an athlete's REE would be of great value.

REE is known to be influenced by age, sex, body size, hormone levels, as well as body composition (33,34). The major determinant of REE is fat-free mass (FFM) (26). Consequently, FFM is routinely used to estimate REE for athletes who have a large FFM to BW ratio. A formula established by the Japan Institute of Sports Sciences utilizes REE per kg FFM and is often employed to estimate REE for athletes (23). However, FFM is not an energetically homogeneous compartment, but rather consists of a variety of heat-producing components (12). The metabolic rate of internal organs can differ substantially. Therefore, FFM may need to be compartmentalized at the organ-tissue level, in order to establish a more accurate estimation of the relationship of FFM to REE.

An advance in this regard was made by Heymsfield et al. (28), who evaluated the contribution of organ-tissue mass on REE using a four organ-tissue compartment. The compartments they utilized were bone mass (BM), adipose tissue (AT), skeletal muscle (SM), and residual mass (RM) which includes internal organs with high metabolic rates. Their subjects were untrained males and females. They found that REE/FFM ratio decreased as FFM increased, due to the fact that the RM to FFM ratio was not constant, but instead decreased with increases in FFM. Because the RM is a more metabolically active compartment than the others, the REE/FFM ratio would decrease as FFM increases, therefore estimating REE based simply on FFM is inadequate. However, their finding was based on untrained

individuals, and it is not clear to what extent their findings would apply to athletic individuals with sport-specific distinct body features. Athletes in general have a body composition characterized by a greater FFM as compared to untrained individuals (30). Therefore, if the REE/FFM ratio is not constant due to the difference in proportion of organ-tissue mass to FFM, the error between measured and calculated REE becomes larger when estimating REE based on FFM.

Taguchi et al. (35) reported that organ-tissue mass is responsible for determine REE for athletes, and thus it can be accurately measured by FFM. The investigator utilized Japanese female athletes over a wide range of body sizes. However, a number of factors that might influence REE, such as average FFM and % body fat, as well as sex hormones (36) between males and females. These are the reason for why the investigation of REE in relation to FFM must be done separately for athletes and untrained individuals, and also for males and females.

Therefore, in this study, the purpose was to evaluate the relationship between FFM and REE at the organ-tissue level by the above approach for male athletes. The working hypothesis was that the REE to FFM ratio would remain constant due to the uniformity of contribution of the organ-tissue masses that make the major contribution to FFM. Thus, the FFM would be a valid predictor for determining the REE of male athletes.

METHODS

Subjects

Fifty seven healthy collegiate male athletes aged between 18-22 yrs participated in this study. They belonged to either the American football (AF) (n=42) or Handball (HB) (n=15) teams. Both teams were ranked within the top three at the National Collegiate championships in 2010. None of the subjects had a history of cardiovascular, endocrine, or orthopedic disorders nor had been taking any

medication when the measurements were taken. Since thyroid hormone influences metabolic rate (37), the blood level of thyroid hormone (triiodothyronine;T_3) was evaluated for each subject using Chemiluminescence Immunoassay (CLIA) technique. The values ranged from 70-176ng/dl, which is within the normal range. The subjects were placed in one of three groups according to their FFM. The groups all consisted of 19 individuals and were termed Small (S), Medium (M), and Large based on the significant differences in FFM between the groups to represent each body size (Table 2-1). The compositions of these groups were as follows; S: AF = 18 and HB = 1; and both M and L: AF = 12 and HB = 7. Subjects were given a verbal and written description of the study and their informed consent was obtained before testing. The study was approved by the Human Research Committee of Waseda University for use of human subjects in accordance with the Declaration of Helsinki.

Body composition measurements

Body weight (BW) was measured to the nearest 0.1 kg by using an electronic scale (Inner Scan BC-660, Tanita Co., Tokyo, Japan). Standing height (Ht) with minimal clothing and with the shoes off was measured to the nearest 0.1cm by using a stadiometer (YL-65, Yamagi, Inc., Nagoya, Japan). The body mass index (BMI) was calculated by dividing BW in kilograms by the square of Ht in meters (kg/m^2).

Dual-energy X-ray absorptiometry (DXA) (Hologic QDT-4500, DXA Scanner, Hologic Inc., Whaltham, MA, USA), a relatively easy and non-invasive technique (38), was used to measure bone mineral content (BMC) (g), lean soft tissue mass (g), and % body fat. FFM and fat mass (FM) were then calculated based on BW and % body fat. Subjects wore loose-fitting light cloth without any metal objects and were positioned supine on the scanning table to perform the total body scan.

REE measurement

REE was measured by open-circuit indirect calorimetry using a Douglas bag. Subjects came to the testing facility in the early morning. In the 12 hr period before testing, no food or liquid (other than water) was consumed (11). The subjects were asked to minimize any exertion prior to the laboratory visit for REE determination. After a 30-40 min period of rest in the supine position with the mask on (Rudolph mask; Hans Rudolph Inc., Kansas City, Mo, USA), two 10-min samples of expired gas were collected in the bags. Resting heart rate and body temperature were measured during the rest period to confirm an adequate duration of the rest period. The laboratory was kept at a neutral temperature (20-25℃) based on the previous report (11), and noise was kept to a minimum. The subjects were instructed to remain awake, quiet, and motionless before and throughout the measuring periods.

Oxygen and carbon dioxide concentrations were analyzed using an expiration gas analyzer (Minato AE-300S, Minato Medical Science, Tokyo, Japan). The volume of expired air was determined by a dry gas volume meter (DC-5, Shinagawa, Japan) and converted to a standard temperature, pressure, and dry condition (STPD). Gas exchange results were converted to REE (kcal/day) using Weir's equation (39). The mean of the two measured values was used for analysis.

Organ tissue mass and estimated REE

The masses of the four organ-tissue compartments were estimated based on values obtained from dual energy x-ray absorptiometry (DXA) using the previously reported prediction model (28) as follows: BM was calculated by multiplying BMC times 1.85. FM was assumed to be 85% of total body AT, therefore AT could be calculated by multiplying FM times 1.18. SM was estimated using the sum of appendicular lean soft tissues and age in a prediction model established by Kim et

al. (40). RM was obtained by subtracting the sum of the calculated BM, AT, and SM from BW, and it included internal organs (i.e. heart, brain, liver, kidneys, spleen, and gastrointestinal tract) as well as skin and glands.

BM (kg) = BMC (g)×1.85/1000

AT (kg) = FM (kg)×1.18

SM (kg) = 1.13×LST (kg)−0.02×age (years)+(0.61×sex : male=1)+0.97

RM (kg) = BW−(BM+AT+SM)

Values for the resting metabolic rate of the each four compartments was adopted from values published in a previous study; 2.3kcal/kg for BM, 4.5 kcal/kg for AT, 13 kcal/kg for SM, and 53 kcal/kg for RM (12). Estimated REE (REEe) was obtained based on the sum of the four body compartments (BM, AT, SM, and RM) by multiplying the corresponding tissue respiration rates as follows:

REEe = 2.3BM+4.5AT+13SM+53RM

Statistical Analysis

The data were expressed as mean ± standard deviation (SD) for all variables. SPSS ver. 17.0 was used for statistical analysis (SPSS Inc., Chicago, IL, USA). The differences among the three body size groups were analyzed utilizing a one-way analysis of variance (ANOVA). The Tukey test was employed to locate the source of the significant differences where appropriate. The level of significance was defined as $p < 0.05$ for all statistical analyses.

RESULTS

Subjects characteristics

The characteristics of the subjects are shown in Table 2-1. The mean age of each body size group tended to increase with increases in body size. Ht was significantly greater in the Large group. BW and BMI significantly increased as body size increased. FM and %body fat were significantly higher in the Large

group.

Measured and estimated REE

The overall average of absolute REE increased significantly in accordance with FFM (Table 2-2 and Figure 2-2). Only the Large group showed a significantly lower REE/BW ratio when compared to the other two body size groups (Table 2-2). However, when REE was divided by FFM, there were no longer significant differences between the three groups. REEm and REEe showed a significant correlation ($r=0.76$, $p>0.001$), and the difference between REEm and REEe was 8± 158kcal.

FFM contribution to REE

The absolute and relative values of each of the four organ-tissue compartments are shown in Figure 2-1. The masses of BM, SM, and RM increased significantly in accordance with larger body size. The average mass of AT was found to be significantly larger in the Large group as compared to the masses of AT in the other two groups. Relative masses of BM and SM were relatively similar between the three groups. On the other hand, AT and RM were higher in the Large group as compared to the Small and Medium groups for AT, and the Medium group for RM. Figure 2-2 shows the energy expenditures in kcal/day of these four organ-tissue compartments. The relative contributions of SM and RM to REE were not significantly different between the groups. Figures 3 and 4 show the relationship between FFM with REEm and REEm/FFM. REEm was highly correlated with FFM ($r=0.84$, $p<0.001$). However, REEm/FFM did not show any correlation with FFM. The contribution percentages of FFM organ-tissue to FFM are shown in Figure 2-5. There were no significant differences in relative contribution of each FFM organ-tissue compartment between the three groups.

DISCUSSION

In the present study, male athletes with a wide range of FFM were utilized to understand the relationship between FFM and REE at the organ-tissue level (Figure 2-1). It was found that, for male athletes, REE/FFM does not change due to the fact that the percentage of organ-tissue mass contribution to FFM is consistent regardless of body size.

Contribution of four compartments on REE

As a result, all four compartments except for AT between Small and Medium groups, increased as body size increased. The four organ-tissue compartments have a specific metabolic rate; BM and AT have relatively smaller weight related metabolic rates (2.3 and 4.5 kcal/kg/day), on the other hand, SM and RM have higher metabolic rates (13 and 53 kcal/kg/day) (28). REEe can be obtained utilizing these metabolic rates with actual mass of each organ-tissue compartments based on the previously established model (35,38). According to the previous studies which used the same organ-tissue REE prediction method, the correlation coefficients between REEm and REEe were 0.75 ($p<0.001$) for untrained males and females, and 0.77 ($p<0.01$) for Japanese female athletes (28,35). On the other hand, the correlation coefficient was 0.76 ($p<0.001$) in the present study. The estimation error in a study by Usui et al. (38) using the same methodology was 19±105 kcal in the group of young high fitness females, in comparison with 8±158 kcal in the present study. Based on these reports, the organ-tissue prediction model is well established method to predict REE and we assumed REE was adequately predicted by using the same method. Therefore, we hold that for male athletes differences in the dependence of REE on body size can be attributed to changes in organ-tissue mass, just like that of untrained individuals and female athletes. RM includes different internal organs such as brain, heart, liver, and kidneys (240, 440, 200, and 440 kcal/kg/day) and these organs have exceptionally high weight related metabolic

rates when compared to the tissues of other organs of the body (12). Thus, RM has highest metabolic rates among the four organ-tissue compartments (53 kcal/kg/day). In fact, RM alone contributes 71% of the total REE (Figure 2-2). The previous study with Sumo wrestlers have shown that the FFM contribution to sum of four organs (brain, heart, liver, and kidney) was 6.3% in comparison with 6.6% for control group, thus the difference was not prominently large. Therefore, it is reasonable to assume that metabolic rate of 53kcal/kg for RM can also apply to the athletes of the present study. On the other hand, although, metabolic rate of SM is much smaller than RM (SM: 13 kcal/kg/day vs. RM: 53 kcal/kg/day), athletes have large absolute mass as in SM. As a result, SM accounts for about 25% of REE, and together, SM and RM account for about 95% of the total REE. As for AT, it increases not just as absolute mass, but also as % AT and %body fat in accordance with increase in body size (Figure 2-3). For this reason, it suggests that athletes with larger body size in regards to FFM tend to have more FM. However, since AT has a relatively low metabolic rate (4.5kcal/kg/day), it has little influence on total REE (3.2% of REE). BM also increases as body size increases without the change in the percentage contribution to BW between the groups (Figure 2-1), but it accounts for less than 1% of REE (Figure 2-2).

Relationship between FFM and REE

In consequence, FFM is largely determined by SM and RM and is thus the major determinant of REE. In the present study, we found that REEm was highly associated with FFM which agrees with the previous studies (12,28,35) (Figure 2-3). FFM consists of metabolically high compartments such as SM and RM, hence REE correlates with FFM. On the other hand, REE/FFM ratio did not change in accordance with FFM (Figure 2-4). This finding is at variance to the results of Heymsfield et al. (28) which indicate that REE/FFM ratio decreases as FFM

increases. They supported their finding based on the decrease of the contribution of RM to FFM as FFM increases. According to Midorikawa et al. (41), the REE/FFM ratio of both controls and Sumo wrestler were the same, even though the difference between the two groups was more than 40kg in BW and 25kg in FFM. This result implies that organ-tissue mass may increase in parallel with FFM, and as expected, it was actually the case that the proportion of BM, SM, and RM to FFM were similar between the groups (Figure 2-5). Therefore, the reason that REE/FFM ratio for athletes was consistent regardless of FFM may be explained by the steadiness of the organ-tissue contribution to FFM. Furthermore, according to Illner et al. (42), even though muscle and organs are constituents of FFM, when compared the correlation coefficients of FFM, muscle mass, and sum of organs with REE, FFM was most associated with REE. If these results are upheld, then FFM is a reliable variable to use when estimating REE for male athletes of all sizes.

Limitations

There are two possible limitations to this study. First, the metabolic rates of each organ-tissue could not be directly measured and was assumed to be constant based on the results of previous studies (28,38,41). A second limitation is the subjects were recruited from a limited set of sports. In order to generalize the findings from this study onto all Japanese male athletes, the utilization of subjects from a broader range of sports would be necessary especially a smaller size athletes.

Conclusion

In conclusion, differences in REE in accordance with body size in male athletes were accounted for by observed differences in organ-tissue mass, especially SM and RM. The proportion of the body organ-tissue compartment was found to be consistent over the utilized range of FFM. This resulted in REE/FFM ratio remaining constant, therefore it is appropriate for FFM to be considered the major

contributor when determining REE for male athletes.

Table 2-1. Subjects characteristics

	All	Small (n=19)	Medium (n=19)	Large (n=19)
	n=57	n=19	n=19	n=19
Age（year)	19.7 ± 1.2	18.8 ± 1.0 *	19.7 ± 0.8	20.5 ± 1.1 †
Height (cm)	175.3 ± 6.4	171.2 ± 5.9	174.8 ± 6.0	180.0 ± 4.1 *†
BW (kg)	78.4 ± 11.5	67.1 ± 4.8 *	77.1 ± 2.9	90.9 ± 8.8 *†
BMI (kg/m^2)	25.5 ± 3.2	22.9 ± 1.5 *	25.3 ± 2.0	28.1 ± 3.4 *†
Body fat (%)	14.2 ± 3.9	12.9 ± 2.4	13.1 ± 3.5	16.5 ± 4.6 †
FM (kg)	11.3 ± 4.7	8.7 ± 1.9	10.2 ± 2.9	15.0 ± 5.8 *†
FFM (kg)	67.0 ± 8.1	58.4 ± 4.1 *	66.7 ± 1.9	75.9 ± 4.6 *†

Values are mean±SD
BW: body weight, BMI: body mass index, FM: fat mass, FFM: fat-free mass,
Significance was determined by one-way analysis of variance (one-way ANOVA)
*significantly different vs Medium ($p<0.05$)
†significantly different vs Small ($p<0.05$)

Table 2-2. Measured and estimated resting energy expenditure (REE)

	All	Small	Medium	Large
	n=57	n=19	n=19	n=19
REEm				
(kcal/day)	1856 ± 225	1643 ± 144 *	1865 ± 140	2060 ± 156 *†
(kcal/kg BW/day)	23.8 ± 1.8	24.5 ± 1.4	24.2 ± 1.8	22.8 ± 1.7 †
(kcal/kg FFM/day)	27.8 ± 1.9	28.1 ± 1.6	28.0 ± 2.3	27.2 ± 1.7
REEe				
(kcal/day)	1848 ± 229	1614 ± 121 *	1855 ± 97	2076 ± 164 *†

Values are mean±SD
BW: body weight, BMI: body mass index, FM: fat mass, FFM: fat-free mass,
Significance was determined by one-way analysis of variance (one-way ANOVA)
*significantly different vs Medium ($p<0.05$)
†significantly different vs Small ($p<0.05$)

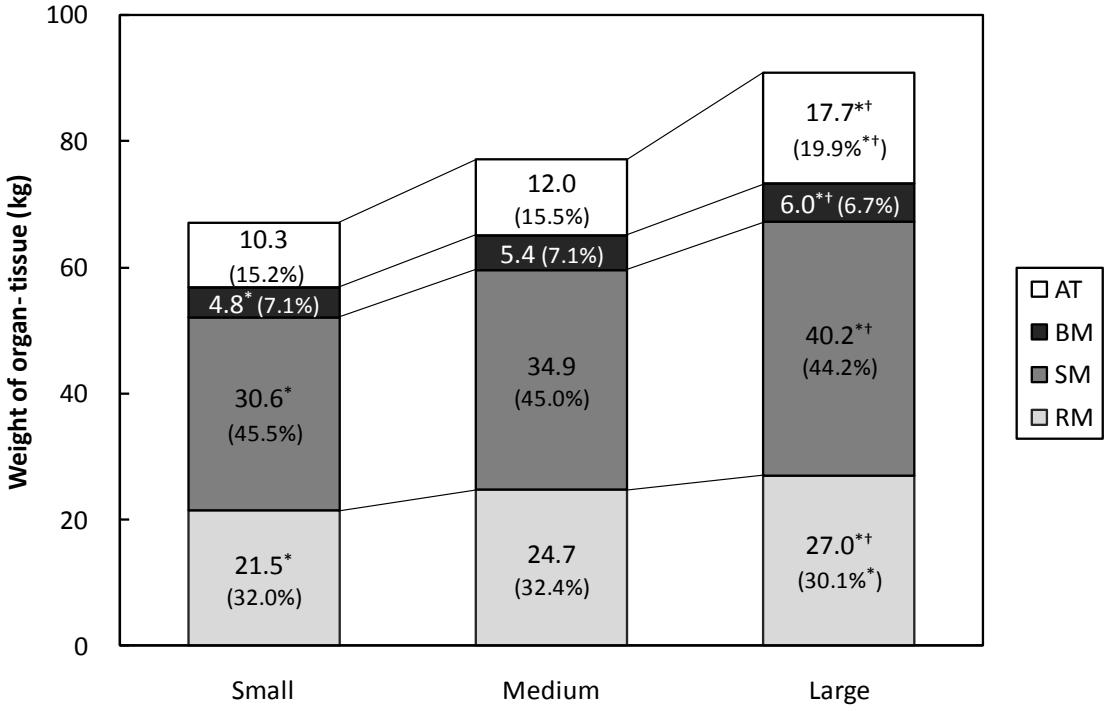

Figure 2-1 Four organ- tissue components expressed as a weight and their respective fractional contribution to BW.

BM: bone mass, AT: adipose tissue, SM: skeletal muscle, RM: residual mass.

*significantly different vs. M ($p<0.05$), † significantly different vs. S ($p<0.005$)

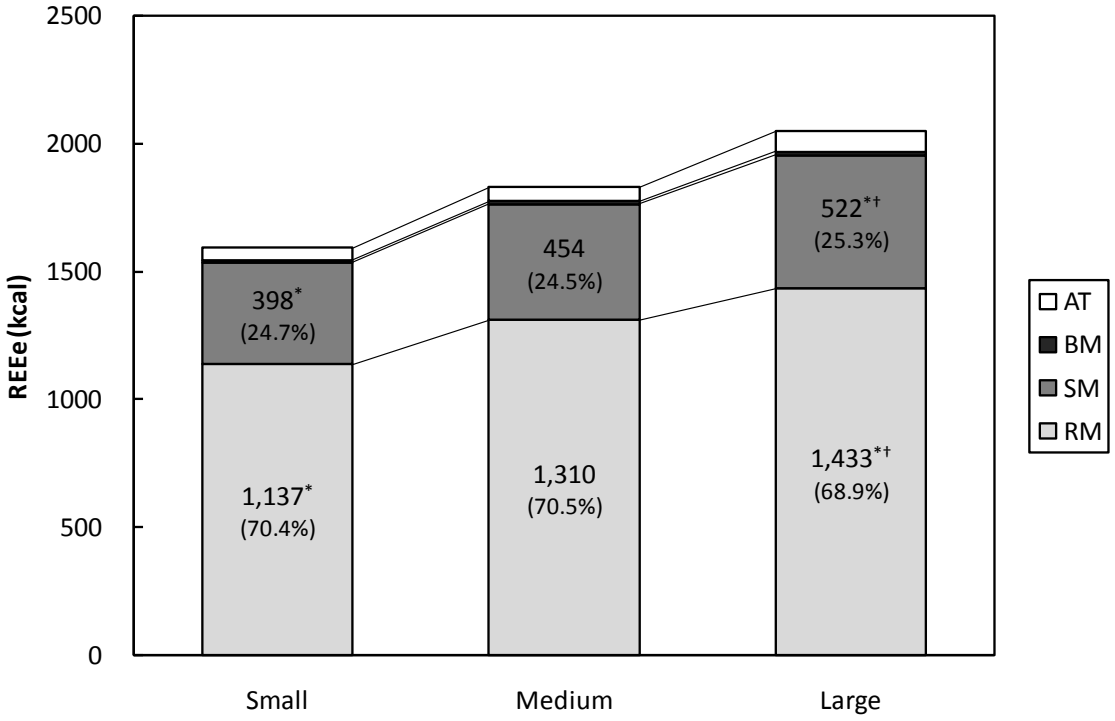

Figure 2-2 Four organ-tissue components expressed as specific energy expenditure and their respective fractional contribution to REEe.

BM: bone mass, AT: adipose tissue, SM: skeletal muscle, RM: residual mass.

*significantly different vs. M ($p<0.05$), † significantly different vs. S ($p<0.005$)

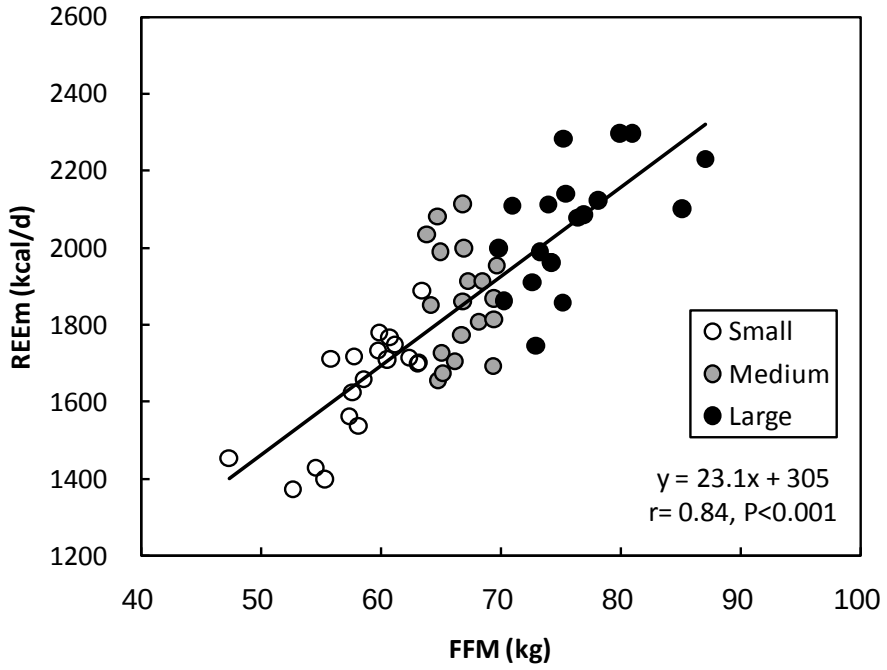

Figure 2-3 Relationship between fat-free mass and measured REE

FFM: fat-free mass, REEm: measured resting energy expenditure

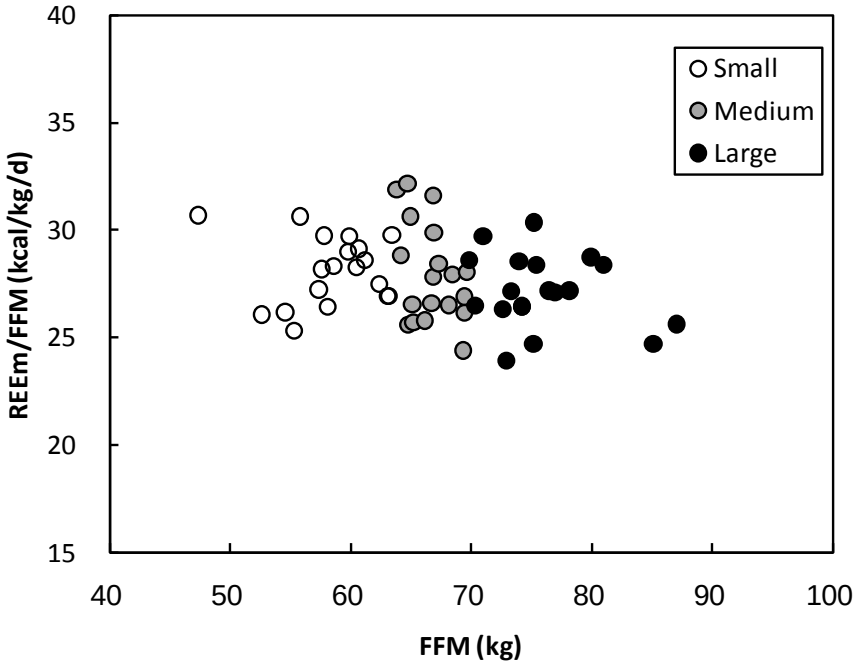

Figure 2-4 Relationship between fat-free mass and measured REE/FFM

FFM: fat-free mass, REEm: measured resting energy expenditure

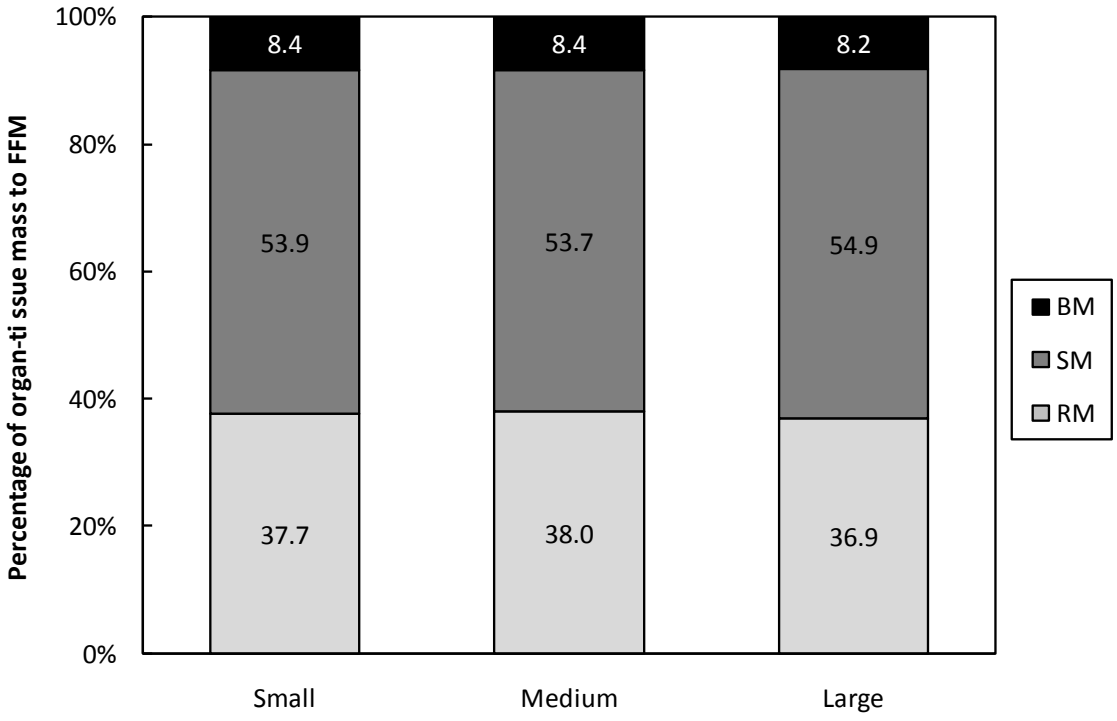

Figure 2-5 Percentage contribution of organ-tissue mass to FFM.

BM: bone mass, SM: skeletal muscle, RM: residual mass.

Chapter 3

Influence of fat-free mass gain on resting energy expenditure in male power athletes

INTRODUCTION

Accurate estimation of total energy expenditure is important for a nutritional assessment and to give an adequate dietary recommendation tailored towards athletes' individual need. Total energy requirement is typically estimated based on the REE multiplied by the appropriate activity factor according to the Dietary Reference Intakes for Japanese, 2010 (DRIs) (8). For athletes, Japan Institute of Sports Science (23) recommends to estimate REE based on the resting metabolic rate of FFM (REE/FFM ratio). FFM is knows for major determinant of REE and athletes typically have higher FFM to FM ratio than non-athletes opponents who have a same body weight. Therefore, utilizing FFM to estimate REE for athletes seems to be reasonable. Our previous study as well as Taguchi et al (35,43) showed that since REE/FFM ratio does not change regardless of different body sizes, FFM can be used to assess REE for both male and female athletes.

FFM is not metabolically homogeneous compartment (25), but rather contains various tissue and organs with specific metabolic rates (12) which varies significantly. On the other hand, according to the previous studies based on general non-athletic subjects, there is a negative relationship between FFM and REE/FFM ratio (20,24,28). Reduction in REE/FFM ratio according with increase in FFM was explained by the decreased contribution of internal organs with high metabolic rate and increase low metabolic rate tissue mass such as skeletal muscle. However, in our previous studies (43) (Chapter 2) indicated that there was no significant correlation between FFM and REE/FFM ratio, suggesting that most of the fractional metabolic contributions on REE from organs and tissue components, skeletal muscle, bone mass, residual mass including organs, were consistent regardless of FFM.

Unfortunately, majority of the studies examined the relationship between FFM

and REE were based on the cross-sectional studies including our study, therefore some other factors may be influencing REE for example body structure such as body height and genetic background. It is known that metabolic rate of body weight is lower with taller subjects compared to shorter subjects. This phenomenon is related to the influence of "surface law" which was first introduced by Rubner in 1883 (13). Moreover, genetic background has known to have influence on REE independent from body composition. It has been reported that polymorphism of uncoupling protein and beta 1, 2, or 3 adrenergic receptor gene are related to metabolic rate (44,45).

A longitudinal study would remove the influence of inter-individual difference such as body structure and genetic profiles and able to capture the clear association between FFM and REE. Additionally, it would provide the useful situational solutions for estimating REE when athletes involve in weight gain. Therefore, the purpose of this study was to enhance our understanding of the relationship between FFM and REE through one year of FFM gain in male power athletes.

METHODS

Study design

Twenty-eight 1st year college American football players who belonged to one of the teams in the National collegiate athletic association division 1 football chamber have participated in the one year longitudinal study. The baseline measurements were taken during June and July of 2010 for sixteen subjects and same time of the year in 2011 for the rest of twelve subjects, and their post measurements were obtained after one year of weight gain period around the same time during the following year. Most of the subjects had already started the daily training with the team for one to two months when the baseline values were measured. The nutritional seminar was held at the beginning of the weight gain period promoting

the effective weight gain especially focusing on skeletal muscle gain. They were told to consume 500-1000 kcal each day in addition to their total energy requirement calculated based on their body composition. The personal nutritional advises were given occasionally for the rest of the period. Subjects have participated in the training six days a week for at least two hours each day. The content of the daily training varies depending on a season and each position that they play. In general, it was in combination of resistance training to hypertrophy skeletal muscle and to increase muscle strength, interval training for improving basic physical performance including running speed and agility, and skill training to improve specific skills required for their specific position.

None of the subjects had a history of cardiovascular, endocrine, or orthopedic disorders nor had been taking any medication when the measurements were taken. Subjects were fully informed about the research study through a verbal and written description. Their informed consent was obtained before testing. The study was approved by the Human Research Committee of Waseda University for use of human subjects in accordance with the Declaration of Helsinki.

Body composition

Body height was measured using the stadiometer (YL-65, Yamagi, Inc., Nagoya, Japan), body weight was measured by the electronic scale using bioelectric impedance technique (Inner Scan BC-660, Tanita Co., Tokyo, Japan), and fat mass (FM) and FFM mass were calculated based on the % body fat measured by the dual energy x-ray technique (DXA) (Hologic QDT-4500, DXA Scanner, Hologic Inc., Whaltham, MA, USA). The four compartments of the body such as bone mass, adipose tissue, skeletal muscle, and residual mass were estimated based on the previously reported formulas (28,46). Please see the method section paragraph "*Organ tissue mass and estimated REE*" in the Chapter 2 for the body composition

measurements and estimation of REE in detail.

Measured REE

REE was measured by open-circuit indirect calorimetry using Douglas bags. Please see paragraph "REE measurements" in the Chapter 2 for the detail procedure of REE measurement.

Biochemical parameters

Whole blood was sampled from a cephalic vein in the morning after at least 12 h of fasting and immediately after REE determination. The blood samples were collected with the volunteers in sitting positions by a certified nurse using 21 or 22 gauge butterfly needles (Terumo corp. Tokyo, Japan) with Luer adapter and tube holder (Terumo corp. Tokyo, Japan). Thyroid hormone (triiodothyronine; T_3) and other health parameters (red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, total and HDL cholesterol, triglyceride, and fasted plasma glucose) were measured by the blood sample taken after more than twelve hours of fasting. The T_3 level of all the subjects was within its normal range determined by the Blood Analysis Laboratory (BML Co. Ltd, Tokyo) except for one subject who could not collect the blood sample at his baseline measurement.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD). SPSS ver. 20.0 was used for statistical analysis (SPSS Inc., Chicago, IL, USA). T-test was used for comparisons between measurements taken at the baseline and after the weight gain. Multiple regression stepwise analysis was used to determine whether the FFM, FM, and plasma T_3 level were independent factors influence REE. The level of significance for all statistical analyses was defined as $p<0.05$.

RESULTS

Subjects characteristics

The average weight gain was 7.4±3.7kg with 9.8% increase after one year of weight gain period. FFM was increased by 3.9±2.1kg (6.0%) and FM gain was 3.5±2.4kg (31.0%) (Table 3-1). Thus 52.7% of total weight gain was accomplished by increase in FFM. The T_3 level stayed within the normal range for all the subjects at both the baseline and after the weight gain.

Measured and estimated REE

The measured REE (REEm) was significantly increased by 74±119kcal (4.2%) after about 10% of weight gain (Table 3-2). The estimated REE (REEe) increased by 97±73kcal kcal/day (5.4%). REEm adjusted by body weight was significantly decreased (1.1kcal/kg BW/day), however, REEm remained stable when REEm was adjusted by FFM. There was no significant difference between REEm and REEe at the baseline, however, the REEe was significantly higher than REEm by 53kcal/day at post-measurement.

FFM components and REE

There were no significant association between FFM and REEm/FFM ratio neither at baseline ($r=0.262$, $p=0.179$) or after 10% of weight gain ($r=0.296$, $p=0.126$) (Figure 3-1). Absolute and relative increase in the mass of four components were 4.1±2.1kg (29.9%) for adipose tissue, 0.2±0.2kg (3.8%) for bone, 2.1±1.1kg (6.3%) for skeletal muscle, and 0.9±1.2kg (4.2%) for residuals (Figure 3-2). Increase in FFM correlated with the change in bone mass ($r=0.427$, $p<0.05$), skeletal muscle mass ($r=0.860$, $p<0.001$), and residual mass ($r=0.806$, $p<0.001$). Skeletal muscle relative contribution to FFM had significantly increased from 53.6% to 54.2%, on the other hand, relative contribution of residual mass significantly decreased from 38.0% to 37.6% (Figure3-3).

Adipose tissue contribution to REE has statistically significantly increased from

60kcal/day to 78kcal/day (30%) (Figure 3-4) The absolute contribution to REE from skeletal muscle and residual mass significantly increased by 27kcal (6.2%) and 50kcal (3.9%), respectively. The fractional contribution of skeletal muscle to REE remains unchanged. On the other hand, the % contribution on REE from the residual mass has decreased significantly from 71.7% to 70.7% one year later.

According to the multiple regression stepwise analysis, at baseline measurement FFM explained 69.8% of variance of REE (Table 3-3). However, there was no other independent factor influencing REE at baseline. After one year later with 10% weight gain, the FFM explained 59.4% of REE.

DISCUSSION

This is the unique study in which we evaluated the influence of FFM gain on REE by actually increasing FFM through a longitudinal study with male power athletes. We investigated the relationship between FFM and REE without the inter-individual difference such as body height by assessing changes of FFM components in the present study.

Changes of FFM components

A 7.4kg (10%) of weight gain in male power athletes led to 3.9kg and 3.5kg increase as FFM and FM, respectively. Furthermore, there was a considerable increase in REEm (74kcal) after one year of weight gain. The increase in REEm was expected since all the absolute organ-tissue mass (bone, adipose tissue, skeletal muscle, and residual mass) increased in accordance with weight gain. Although the resting metabolic rate per body weight was significantly decreased after the weight gain, REE/FFM ratio did not change from the baseline measurement and to one year later (27.3kcal/kg/day to 26.9kcal/kg/day). These findings matched the findings in our previous studies (43) (Chapter 2) in which REE adjusted by FFM did not change regardless of FFM in male athletes. This suggests that FFM, rather

than body weight, is better parameter to utilize for estimating REE for male athletes.

We also found in our previous study (43) (Chapter 2) that the consistency of REE rate to FFM was the result of the constant relative contribution of FFM components (bone mass, skeletal muscle mass, and residual mass) to total FFM regardless of their FFM. On the other hand, there were statistically significant but small changes in the relative contribution of these three FFM components in the present study from the baseline to one year after the measurement. The relative contribution of bone mass remained unchanged. On the other hand, skeletal muscle relative contribution to FFM increased by 0.8% and residual mass contribution has decreased by 0.4%. Logically these changes reduces the REE/FFM ratio since the changes were the reduction of proportional contribution of metabolically high component, residual mass (54kcal/kg/day), and increase in relatively low metabolic rate component, skeletal muscle (13kcal/kg/day). Heymsfield et al. (28) proposed the four compartment models which can estimate REE to investigate the rational for the inconsistency of REE/FFM ratio between the subjects with small and large FFM. They found that the low REE/FFM ratio with large subjects was due to the low proportion of FFM as residual mass and high proportion as skeletal muscle and bone. Illner et al. (42) also suggested, in the study with twenty-six non-obese adult male and female, which the decrease in measured REE to FFM ratio with increasing FFM was associated with increasing ratio of total skeletal muscle mass to sum of organ mass. The range of FFM in the previous cross-sectional studies were much larger compared to the increase of FFM seen in the present study. Therefore, if the increase of FFM is larger than 3.9kg, the changes of FFM components may actually lead to a significant reduction of REE/FFM ratio similarly to the previous studies.

Role of residual mass

Among the four components, residual mass is the only tissue which contains variety of organs and tissue including blood and glands. It is unknown that if all of these organs and tissue among residual mass proportionally increase as FFM increases, and if it changes then it is possible that the metabolic rate of residual mass changes. According to the previous study, the relative contribution of liver consistently contributed to FFM throughout the different FFM, however brain mass negatively correlated in the fraction to FFM (28,47). Therefore, if the relative contribution of organs such as brain to residual mass changes, the metabolic rate of residual mass possibly changed since brain mass probably does not change no matter the FFM was gained. In fact, REEe was significantly overestimated by 53 kcal/day compared to the REEm at the post-measurement in the present study. Unfortunately, due to the difficulty of directly measuring metabolic rates of each organs and tissue, the metabolic rates of individual organ-tissue were assumed to be the same as the previous study and used the same values as previous study reported. Therefore, this may be the cause of difference between measured and estimated REE after one year of weight gain period. In any cases, it would be helpful to assess the actual changes of organ mass with weight gain and examine its influence on REE in power type athletes as a future study. Especially, power type athletes commonly involve in resistance training and overfeeding to increase skeletal muscle, therefore, there may be some cases of significant increase in organs mass which may not be able to see in non-athletic person. For example, it is well known that left ventricular hypertrophy by resistance training (48) and also there was the study reporting enlarged kidneys due to the high protein diet (49).

Weight gain and REE

According to the result of multiple regression analysis with stepwise entry,

69.7% of variance of REE was able to explain by FFM at baseline, however after the 10% weight gain, FFM was only able to explain 59.4% of REE. This significant drop (⊿10.3%) of FFM contribution on the variance of REE was perhaps partially able to explained by the remarkable increase of FM in addition to FFM gain. Another reason perhaps could be the change in proportional ratio of FFM components after the weight gain. The result may be suggesting the individual different in the influence of FFM on REE. Bosy-Westphal et al. (29) reported that there is an individual difference in weight loss-associated adaptive thermogenesis. In their study, forty-five obese women (26-46y) followed by the low calorie diet for about 12 weeks and found that women with high adaptive thermogenesis defined by low REEm-REEe difference, had less weight loss and conserved more FFM, such as liver and kidneys, however women with low adaptive thermogenesis lost more weight and FFM as well as liver and kidneys. Therefore, it suggested that some people metabolically adapt differently to the change in body weight.

Interestingly, T_3 became an independent factor influencing REE at post-measurement in addition to FFM. Based on the previous study, the metabolic adaptation to weight change was caused not only by change in body composition but also with effect of thyroid hormone (50). The level of thyroid hormone is thought to relate to sympathetic nerve system (SNS) activity (51). Therefore, although the increase of T_3 level in correlation with body weight gain was not observed, the REE of athletes seemed to have significant influence from thyroid hormone after the weight gain, possibly by the influence from a SNS activity.

Limitation

One limitation of the present study was the total increase of FFM was relatively small (3.9kg), and it was difficult to observe the significant changes in FFM components. As a result, it was difficult to conclude the true influence of FFM gain

on REE/FFM ratio because the relative changes in FFM compositions were small. Therefore, the future study for investigating organs mass with larger FFM gain would likely to provide further understanding regarding the rationale for the influence of FFM gain on REE for male athletes.

Conclusion

In conclusion, we investigated the relationship between FFM and REE through one year of FFM gain in male power athletes, and found that even though there was a 7.4 kg (10%) of body weight gain with 4kg of FFM gain, there were not large changes in the relative contribution of organs-tissue components to FFM. Therefore, REE/FFM ratio did not change with 3.9kg of FFM gain in male power athletes.

Table 3-1. Subjects characteristics

n=28	Baseline	One year later	⊿	p value
Height (cm)	174.4 ± 5.9	174.5 ± 6.0	0.1	0.643
Body weight (kg)	75.8 ± 12.4	83.2 ± 12.8	7.4	<0.001*
Body fat (%)	14.4 ± 3.9	17.3 ± 4.1	2.9	<0.001*
FM (kg)	11.3 ± 5.1	14.8 ± 5.7	3.5	<0.001*
FFM (kg)	64.5 ± 8.0	68.4 ± 7.7	3.9	<0.001*
Adipose tissue mass (kg)	13.4 ± 6.0	17.4 ± 6.7	3.0	<0.001*
Bone mass (kg)	5.2 ± 0.6	5.4 ± 0.6	0.2	<0.001*
Skeletal muscle mass (kg)	33.5 ± 4.2	35.6 ± 4.3	2.1	<0.001*
Residual mass (kg)	23.7 ± 2.6	24.7 ± 2.5	1.0	0.001*

Values are mean±SD, *p<0.05
FM: fat mass, FFM: fat-free mass

Table 3-2. Measured and calculated resting energy expenditure (REE)

n=28	Baseline	One year later	⊿	p value[a]
REEm (kcal/day)	1759 ± 217	1833 ± 207	74	0.003*
(kcal/kg BW/day)	23.4 ± 2.1	22.3 ± 2.2	-1.1	0.003*
(kcal/kg FFM/day)	27.3 ± 1.9	26.9 ± 2.0	-0.4	0.183
REEe (kcal/day)	1790 ± 217	1886 ± 208	96	<0.001*
p value[b]	0.199	0.047*		

Values are mean±SD, *p<0.05
BW: body weight, FFM: fat-free mass,REEm: measured REE, REEe: estimated REE
[a] p value for t-test between baseline vs. one year later
[b] p value for t-test between REEm vs. REEe

Table 3-3. Multiple linear regression stepwise analysis with resting energy expenditure (REE) as the dependent variable and FFM, FM, and T_3 level as the independent variables.

REE (kcal/day)	Baseline			One year later		
	R^2	β	P value	R^2	β	P value
Model 1	0.697			0.594		
Constant						
FFM (kg)		0.835	<0.001		0.771	<0.001
Model 2				0.667		
Constant						
FFM (kg)					0.701	<0.001
T_3 (ng/dL)					0.280	0.027

FFM: fat-free mass, FM: fat mass, T_3: plasma triiodothyronine level (thyroid hormone)

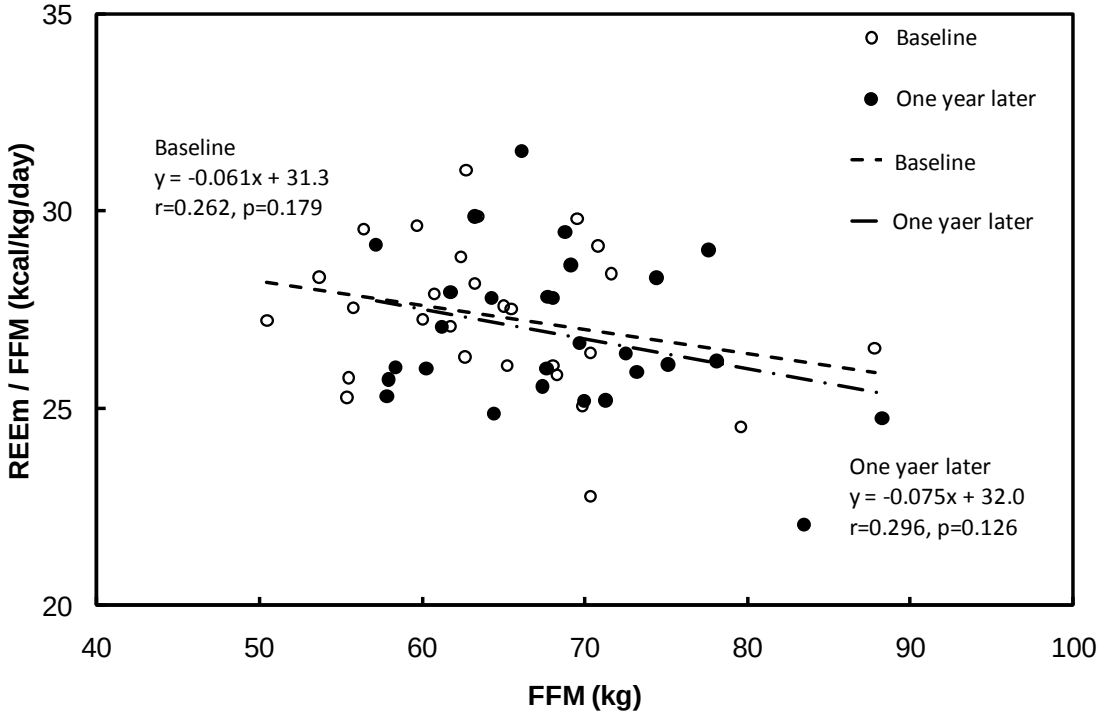

Figure 3-1 Relationship between fat-free mass and measured REE/FFM ratio

FFM: fat-free mass, REE: resting energy expenditure

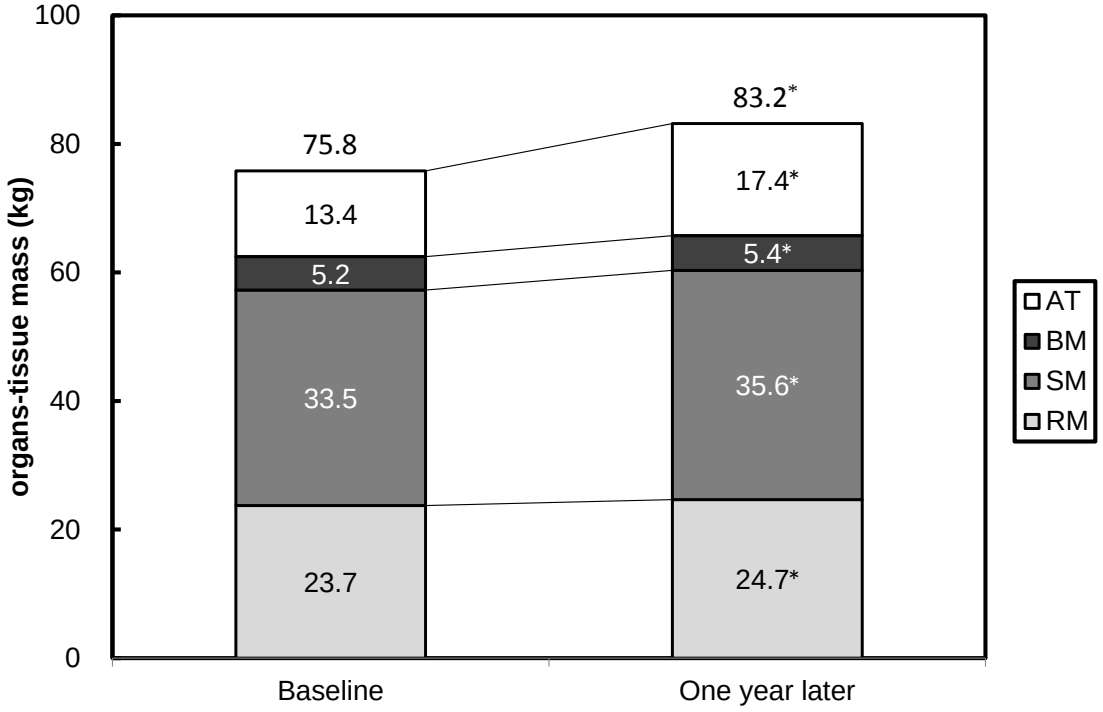

Figure 3-2 Four organ- tissue components expressed as a weight.

BM: bone mass, AT: adipose tissue, SM: skeletal muscle, RM: residual mass.

*significantly different ($p<0.05$)

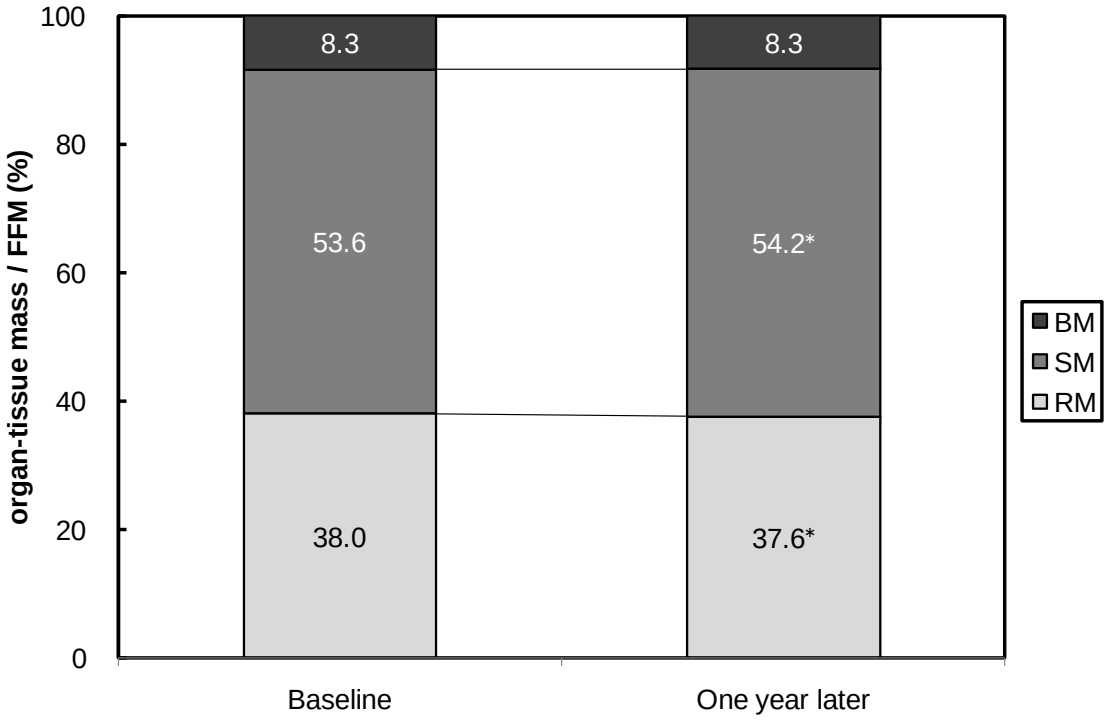

Figure 3-3 Percentage contribution of organ-tissue mass to fat-free mass.

FFM: fat-free mass, BM: bone mass, SM: skeletal muscle, RM: residual mass.

*significantly different ($p<0.05$)

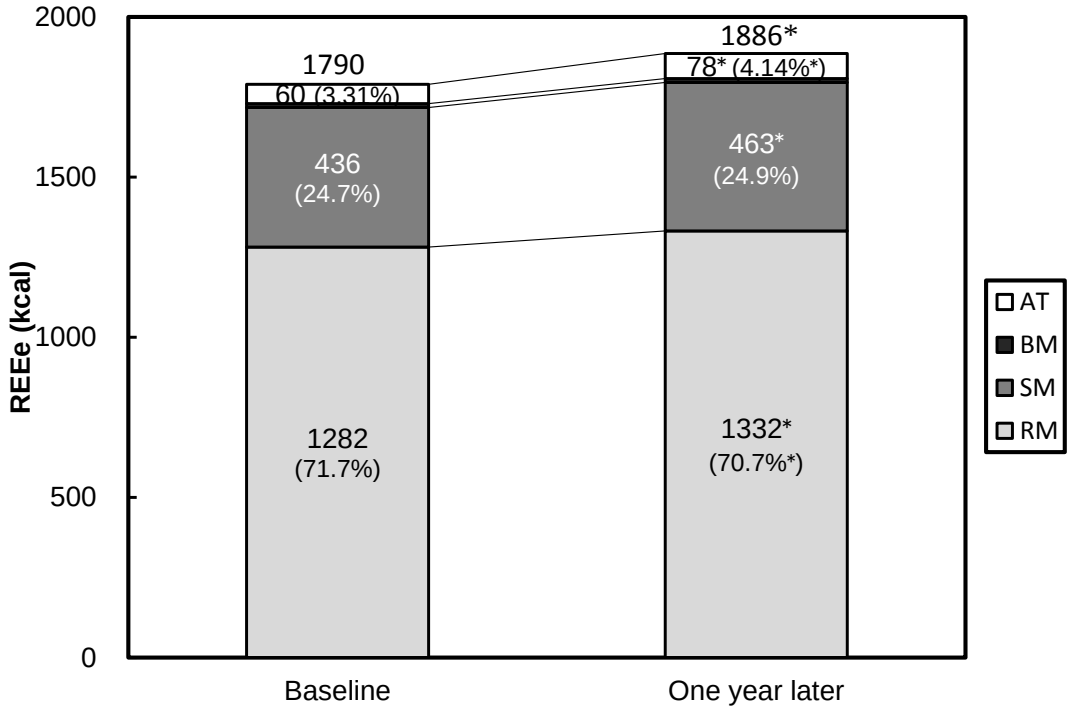

Figure 3-4 Four organ-tissue components expressed as specific energy expenditure and their respective fractional contribution to REEe.

BM: bone mass, AT: adipose tissue, SM: skeletal muscle, RM: residual mass.

*significantly different ($p<0.05$)

Chapter 4

Influence of organs mass on resting energy expenditure in male power athletes

(J Nutr Scie Vitaminol, Under first revision)

INTRODUCTION

Fat-free mass (FFM) is the sum of a variety of tissue and organs with both high and low levels of metabolic activity (12). Previous studies have shown that FFM accounts for 60 to 80% of the variance in REE in non-athletic populations (28,42,52). This seems to be also true of female athletes (35) or women with high physical fitness (38). Typically, athletes will have relatively large FFM compared to non-athletic individuals. Therefore, for athletes, the Japan Institute of Sports Science (JISS) and other study have recommended that REE is estimated by FFM multiplied by the REE to FFM ratio (REE/FFM ratio) (23,53). In order to use FFM to estimate REE for athletes, another assumption that should be confirmed is that the REE/FFM ratio should be constant regardless of individual variations in FFM. Otherwise, there will be tendencies to over- or under-estimate REE among athletes depending on the value of FFM. In fact, there are numerous studies with non-athletic populations, mostly including young to elderly and both males and females with wide ranging of FFM values that have reported that the REE/FFM ratio decreases with increasing FFM (12,20,24,25,54). In addition, the cause for the reductions in REE/FFM ratios appears to be a reduced contribution of the components of FFM with high metabolic activity, such as internal organs, as the FFM increases (28).

Our previous study demonstrated the consistency of the REE/FFM ratio in male college athletes (43). FFM values were divided into 3 components, bone mass, skeletal muscle, and residual mass, with specific metabolic rates previously published by Heymsfield et al. (28) used to investigate the relative contributions of these 3 components of FFM. The results suggested that the percent contribution of each component to FFM did not change regardless of different FFM values.

The main component of FFM is skeletal muscle, which is about 40 to 60% of body weight according to previous studies (43). However, skeletal muscle accounted for only 20 to 30% of REE because the metabolic rate of skeletal muscle is 13 kcal/kg/day, whereas the metabolic rates of liver, brain, heart, and kidney mass were determined to be 200, 240, 440, and 440 kcal/kg/day (12). Thus, these 4 organs account for 60 to 70% of REE in adults, even though their combined weight is less than 6% of total body weight (12). As a consequence, small relative differences in fractional contributions of organ mass to FFM may significantly influence REE/FFM ratios. Moreover, one of the distinct characteristics of power athletes is that their attempts to build an ideal body composition are focused particularly on building skeletal muscle by a combination of diet and daily physical training. The definition of power athletes is athletes who participate in a sport which rely heavily on a muscle strength and power, and require minimum level of endurance ability for their sport. In the previous study with Sumo wrestler (body weight:109.1±14.7kg, FFM:78.6±9.7kg) showed much larger organ mass compared to non-athletic subjects (41). On the other hand, there were no significant difference between the organ mass of obese subjects (body weight:105.4±10.8kg, FFM:66.7±12.5kg) and normal to overweight subjects (29). Hence, there may be a difference to the influence of organ sizes when athletes becoming larger with special emphasis on gaining FFM compared to non-athletes becoming obese.

The purpose of this study was to examine the relationship between organs mass to FFM and its contribution of the internal organs to the REE/FFM ratio among male power athletes. We hypothesized that the reason athletes maintain a constant REE/FFM ratio regardless of FFM is that the relative contribution of the internal organs to the FFM remained unchanged, which proved true for all of the organs of interest, except the brain.

METHODS

Subjects

The study included 37 male American football players from National Collegiate Athletic Association Division 1 teams. There were four 1st year students, twenty-five 2nd year students, and eight 3rd year students who belong to the college level American football team. There was a variety of positions played by the subjects in the sport. All of the subjects were very active since they were young and had participated in some types of sports team since they were in elementary school according to the exercise history survey. The measurements were taken during their off-season. All of them voluntarily agreed to participate in the study. None had a history of cardiovascular, endocrine, or orthopedic disorders, nor had any of them been taking any medication when the measurements were taken. Each subject was fully informed about the research study by verbal and written descriptions, each gave informed consent before testing, and the study was approved by the Human Research Committee of Waseda University for use of human subjects in accordance with the Declaration of Helsinki.

Body composition

Body weight (BW) was measured to the nearest 0.1 kg by bioelectrical impedance analysis (Inner Scan BC-660, Tanita Co., Tokyo, Japan) and standing height was measured to the nearest 0.1 cm with a stadiometer (YL-65, Yamagi, Inc., Nagoya, Japan). Dual-energy X-ray absorptiometry (DXA) (Hologic QDT-4500, DXA Scanner, Hologic Inc., Waltham, MA, USA) was used to measure the appendicular lean soft tissue mass (ALST) (g), and % body fat. FFM and fat mass (FM) were then calculated based on BW and % body fat. Skeletal muscle (SM) mass was estimated using the sum of appendicular lean soft tissues with age in a prediction model established by Kim et al. (40) as follows: SM (kg) = 1.13 × ALST (kg) − 0.02 × age

(years) + (0.61 × sex : male = 1) + 0.97. Adipose tissue is calculated as follows: adipose tissue (kg) = FM (kg) × 1.18. Please see "*Body composition measurements"* in Chapter 2 as a reference for the details.

Organ mass

The volumes of liver, brain, and kidneys were measured using magnetic resonance imaging (MRI) (Signa 1.5T; General Electric Co., Milwaukee, WI). The images were taken by a T1-weighted spin-echo and axial-plane sequence with 10 mm slice thickness, 500 ms repetition time, and 13.1 ms echo time. Brain images for some of 1st year players were measured using a T1-weighted spin-echo with head coil, while the method used in others measured the anterior commissure-posterior commissure plane sequence with a slice thickness of 5 mm, 1.5 mm inter-gap, a repetition time of 500 ms, and an echo time of 14 ms. The coefficient of variation was 0.2% between the 2 brain scanning techniques. The subjects were in the supine position with the hands placed on the abdomen and the legs extended during both MRI techniques. During the scans of the trunk region, in order to minimize blur in the images, the subjects were asked to inhale hold their breath for about 28 s and to breathe when prompted by an announcement. The MRI cross-sectional images of the liver, kidneys, and brain were analyzed using image analysis software (Slice-o-matic; Tomovision, Montreal, QC, Canada), and automatic segmentation of an image was performed using watersheds of the gradient magnitude using the Mathematical Morphology ("Morpho") mode. Cross-sectional areas (cm^2) were determined after assigning areas of interest different color codes for the tissues to be analyzed. The volume of each organ was determined from the sum of its cross-sectional areas multiplied by the 1cm slice thickness. Volumes (cm^3) of the organs were then converted to mass in kg using densities of 1.060 kg/cm^3 for liver, 1.036 kg/cm^3 for brain, and 1.050 kg/cm^3 for kidneys, which were reported

previously (55). All analyses were performed by the same investigator to minimize analysts' variation, and the intra-observer coefficients of variation were 5.2% for liver, 0.6% for brain, and 5.5% for kidneys.

Left ventricular mass (LV mass) was measured using echocardiography (Titan, SonoSite, WA, USA) with 2.8 MHz probe (C15/4-2 MHz, SonoSite, Inc. WA, USA). Subjects were asked to lie in a partial left decubitus or supine position during the measurement. At least 5 M-mode end-diastolic phase images of left ventricle in the parasternal long axis view were captured for the measurements. The dimensions and the wall thickness were evaluated at or below the tips of the mitral valve leaflets. The LV mass was calculated using the 2-dimensional linear formula suggested by the American Society of Echocardiography as follows: LV mass = 0.8 × {1.04 [(LV internal dimension at end diastole + LV wall thickness at the inferolateral walls + LV wall thickness at the cardiac base for the anteroseptum)^3 − (LV internal dimension at end diastole)^3]} + 0.6 g (56,57). This was multiplied by a factor of 1.50 to obtain a mass of total heart (58). The intra-observer coefficient of variation for LV mass was 5.2%.

Measured REE

REE was measured by open-circuit indirect calorimetry using Douglas bags. Please see paragraph "REE measurements" in the Chapter 2 for the detail procedure of REE measurement.

Biochemical parameters

All of the blood sample analyses were conducted in the blood analysis laboratory (BML Co. Ltd, Tokyo) including red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, total and HDL cholesterol, triglyceride, and fasting plasma glucose. Because thyroid hormone influences metabolic rate (37), the blood level of thyroid hormone (triiodothyronine:

T_3) was also evaluated. T_3 is an activated form of thyroid hormone that is necessary for the thyroid hormone action. The T_3 levels of all of the subjects were confirmed to be within the normal range. Please see paragraph "*Biochemical parameters*" in Chapter 3 of the methodology section for the details of the measurements.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD) with the normal range provided in parentheses. SPSS ver. 20.0 was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Multiple regression analysis with forced entry was used to determine whether FFM, FM, T_3 level, and internal organs independently influenced REE. The level of significance for all statistical analyses was defined as $p < 0.05$.

RESULTS

Subject characteristics

Table 4-1 shows the characteristics of the subjects. The average body height was 174.7 ± 5.9 cm, BW was 81.2 ± 11.3 kg, and % body fat was 16.3 ± 4.1%. The largest internal organ was the liver, followed by brain, kidneys, and heart (1.74 kg, 1.40 kg, 0.40 kg, and 0.31 kg). The sum of the 4 internal organs was 3.8 ± 0.4 kg, which was about 4.8% of the total body weight on average. Furthermore, the average relative contribution of the sum of internal organs was 5.7% of FFM. On the other hand, skeletal muscle had the largest contribution to FFM, which was 52.6%. The measured REE was 1869 ± 230 kcal/day and REE/FFM ratio was 27.7 ± 1.9 kcal/kg/day. The measured REE showed a significant correlation with FFM ($r = 0.825$, $p < 0.001$). However, when REE was adjusted by FFM, it did not show a correlation with FFM.

Based on the biochemical parameters none of the subjects were anemic. The average values of total cholesterol, HDL cholesterol, triglyceride, and FPG were all

within normal range. The average T_3 was 128 ± 19 ng/dL and T_3 levels for all of the subjects fell in the normal range.

Organ mass and FFM

There were significant association between all 4 organs and FFM (Figure 4-1 and Table 4-2). Liver showed the highest correlation of all 4 internal organs with FFM ($r = 0.712$, $p < 0.001$). Only the brain showed relatively low correlation with FFM ($r = 0.333$, $p = 0.044$). Furthermore, unlike the other 3 organs, brain did not show a significant association with skeletal muscle. FM was strongly associated with both FFM ($r = 0.637$, $p < 0.001$) and skeletal muscle ($r = 0.522$, $p < 0.01$). Liver, heart, and kidneys significantly related to each other, while brain did not correlate with any of the other organs.

Organ mass contribution to REE

The masses of liver, heart, kidneys, and skeletal muscle all contributed consistently to REE regardless of different FFM (Figure 4-2). On the other hand, the relative rate of brain contribution to measured REE became significantly smaller as the FFM became larger ($r = -0.672$, $p < 0.001$). The averages of the relative contribution to REE from each tissue and organ were: skeletal muscle 24.8 ± 1.8%, liver 18.7 ± 2.1%, brain 18.1 ± 2.1%, heart 7.3 ± 1.2%, and kidneys 9.4 ± 1.8%. The average percentage of the sum of liver, brain, heart, and kidneys to total REE was 53.5 ± 3.9%. The measured adipose tissue also increased as FFM increase ($r = 0.637$, $p < 0.001$), and thus, the relative contribution of adipose tissue to REE became larger as FFM increased ($r = 0.399$, $p < 0.05$). There was also a significantly positive relationship between T_3 and REE, even after adjusting for FFM ($r = 0.457$, $p < 0.01$). Multiple regression analysis was used to assess the effects of FFM, FM, and T_3 on variance of REE, and also the associations between REE and the mass of each organ (kg) after adjustment for FFM, FM, and T_3 (Table

4-3). The results showed that FFM alone explained 68.1% of the variance in REE, and T_3 explained an additional 8% of the variance of REE. FM was not an independent factor influencing the variance of REE. None of the organs significantly influenced REE as independent variables, but liver showed a positive trend ($p = 0.068$) as an independent factor and accounted for an additional 2.5% of variance of REE.

DISCUSSION

The present study has determined that the major reason that the REE/ FFM ratio was steady in the male power athletes was because of the consistency of the contributions of internal organs on REE with high metabolic activity, except for brain.

Organs and FFM

The mass of liver, heart, and kidneys correlated significantly with FFM, and thus the relative contribution of these three organs did not change, regardless of FFM. Similarly, Illner et al. (42) and Sparti et al. (59) found significant correlation between FFM and liver, heart, and kidneys. Heymsfield et al. (47) also reported significant correlation between liver mass and FFM. Furthermore, previous studies have reported correlation between FFM and liver, heart, and kidneys in both young adult men and women, as measured by same method as used in the current study (42,52,59). It is well known that left ventricular hypertrophy with resistance training is associated with increases in left ventricular mass (48,60). Therefore, it may be possible that the relatively lower correlation between FFM and heart in the present study as compared to liver or kidneys is due to the characteristics of the athletes, who were involved in daily physical training, including resistance training. In any case, our results have supported the results of previous research in which liver, heart, and kidneys typically become large correspondently with FFM, no

matter whether the subjects are athletes or non-athletes.

As for brain, there have been other studies that also supported a significant relationship between brain mass and FFM (47,52). Heymsfield et al. (47) reported that human brain positively correlated with FFM, even though the relationship was much weaker than that of liver. They also found that FFM accounted for 55% of the variation of liver mass in men, but only 6% of mass of brain after adjusting for age (47). Illner et al. (42) also found a relatively lower correlation between brain and FFM compared to liver, heart, and kidneys. Based on these findings, it seems that brain does correlate positively with FFM, but the relationship is not as strong as the other internal organs. In the present study, the inclusion of subjects with much larger FFM compared to the previous study (47) may be another reason why the percentage contribution to REE from brain mass was negatively correlated with FFM.

Adipose tissue and REE

Because of the reduced relative contribution of the brain to the REE, REE/FFM could be expected to become smaller as FFM become larger, but in fact, in our subjects, the REE/FFM remained constant, regardless of the FFM. The influence of adipose tissue was considered one of the reasons for this. FFM and adipose tissue were significantly correlated ($r = 0.637$, $p < 0.001$) in the present study, and thus the fractional contribution of adipose tissue to REE increased as FFM became larger ($r = 0.399$, $p < 0.05$), unlike previous studies, which did not show a relationship between FFM and FM (42,59,61). Therefore, adipose tissue may contribute more to increase the REE/FFM ratio in athletes with larger FFM. Male athletes commonly have relatively low percentages of body fat compared to non-athlete males with similar body weight, however, for power athletes in particular with large FFM, FM may be relatively large, and this may result in a marked impact on raising the

REE/FFM ratio.

Factors influence REE

Multiple regression analysis revealed FFM as the major determinant of variance of REE (68.1%) in the present study. However, FM was not an independent factor, and only an additional 0.2% of variability was explained by FM, compared to the model of FFM alone. One of the reasons for the low contribution of FM to the variance of REE is the low metabolic rate of adipose tissue (4.5 kcal/kg/day). T_3 explained additional 7.8% of variability in REE after FFM and FM were adjusted, and its contribution was quite high compared to other studies. For example, a study by Taguchi et al. (22) found that T_3 accounted for 5.3% of REE after adjusting for FFM ($R^2 = 0.503$), similar to the findings of Svendsen et al. (62), in which T_3 explained an additional 2% of REE after adjusting FFM, FM, and androstenedione (precursor of male and female sex hormones) ($R^2 = 0.46$). Thyroid hormone has been found to alter the behavior of many metabolic pathways that are possibly relevant for the basal metabolic rate (63). Strong candidates for the underlying mechanisms are uncoupling of cellular metabolism from adenosine triphosphate (ATP) synthesis, and/or changes in the efficiency of metabolic processes downstream from the mitochondria (63-65). Even though the underlying pathways of thyroid hormone are not fully understood, the energy availability for conversion into fat is reduced by increasing REE. Thus, alterations of thyroid hormones are thought to be an adaptation process to prevent from obesity (66). In the present study, the level of T_3 had a significant association with REE/FFM ($r = 0.463$, $p < 0.05$), despite the fact that T_3 concentrations of all the subjects were within the normal range. Based on these results, although the true factors influencing the level of T_3 in the present cross-sectional study were unknown, thyroid hormone was considered another independent factor that significantly on influences REE/FFM in male athletes.

Finally, none of the organs independently influenced the variance of REE after adjusted FFM, FM, and T_3, although there is some suggestion that liver alone may contribute to the variance of REE ($p=0.068$) after adjustment for FFM and T_3. Since brain was not an independent factor which influencing REE, the reduction in brain contribution to FFM as FFM become larger does not seem to have large impact on REE/FFM ratio. Consequently, the results indicate that even though organ mass is considered, it may not significantly improve the accuracy of predicting REE for male athletes. Hence, the FFM alone should be adequate component among body composition to determine REE for power athletes.

Limitations

There are some limitations in this study. An assumed metabolic rate of organs and tissues was used to calculate the metabolic contributions to REE. However, the metabolic rates were determined based on a Western population (12). Hence, racial variations in metabolic rates may have been an influence. Gallagher et al. (67) have indicated that differences of REE between Caucasians and African Americans were due to the difference in the size of internal organs, but not due to the changes in the metabolic rates (67). For that reason, it is unlikely that the metabolic rates of organs and tissue significantly different between races.

The second limitation was the limited number subjects from different types of sports. All of the subjects of present study were American football players, consequently, there are unknown possibilities for bias in the results. American football is a unique sport with 8 basic positions (68), and in order to carry out tasks of each position on the field, body composition and physical talents of players varies widely (68). This relatively a wide range of body composition, including FFM, was expected to minimize the bias in comparison with male athletes in general. Tatsuta et al. (69) reported that a group of elite athletes with relatively small FFM (average

54.1 ± 3.9 kg) had significantly higher metabolic rates of FFM (31.9 ± 2.6 kcal/kg/day) compared to a group with larger FFM (27.6 ± 2.6 kcal/kg/day). Therefore, additional research with different types of athletes with leaner body compositions, would certainly be helpful in order to confirm the appropriate application of FFM metabolic rates to estimate REE for all types of athletes with wide ranging body compositions.

Conclusion

The study examined the contribution of the internal organs to the REE/FFM ratio among male power athletes and found that the consistency of the REE/FFM ratio regardless of FFM was maintained mostly by the steady relative contribution of internal organs to REE, except for brain.

Table 4-1. Subjects characteristics

n=37	Mean ± SD	Range
Age (year)	20.0 ± 1.0	(18.0 - 22.0)
Height (cm)	174.7 ± 5.9	(161.4 - 184.6)
Body weight (kg)	81.2 ± 11.3	(63.7 - 106.0)
Body fat (%)	16.3 ± 4.1	(7.7 - 26.5)
FM (kg)	13.5 ± 5.0	(5.6 - 28.1)
FFM (kg)	67.7 ± 7.4	(56.9 - 84.2)
Skeletal Muscle (kg)	35.6 ± 4.2	(29.8 - 46.1)
Liver (kg)	1.74 ± 0.28	(1.33 - 2.50)
Brain (kg)	1.40 ± 0.10	(1.18 - 1.61)
Heart (kg)	0.31 ± 0.06	(0.21 - 0.42)
Kidneys (kg)	0.40 ± 0.06	(0.31 - 0.57)
Skeletal Muscle/FFM (%)	52.6 ± 2.4	(49.3 - 60.6)
Liver/FFM (%)	2.6 ± 0.3	(2.0 - 3.2)
Brain/FFM (%)	2.1 ± 0.2	(1.7 - 2.6)
Heart/FFM (%)	0.5 ± 0.1	(0.3 - 0.6)
Kidneys/FFM (%)	0.6 ± 0.1	(0.5 - 0.8)
REEm (kcal/day)	1869 ± 230	(1463 - 2298)
(kcal/kg BW/day)	23.2 ± 2.0	(20.0 - 27.1)
(kcal/kg FFM/day)	27.7 ± 1.9	(24.9 - 32.1)

FM: fat mass, FFM: fat-free mass, REEm: measured resting energy expenditure

Table 4-2. Pearson correlation coefficients among body compartment sizes

n=37	FFM	FM	SM	Liver	Brain	Heart
FFM						
FM	.637**					
SM	.917**	.522**				
Liver	.712**	.646**	.628**			
Brain	.333*	.050	.250	.215		
Heart	.538**	.469**	.470**	.493**	-.106	
Kidneys	.683**	.606**	.610**	.709**	.105	.494**

FFM: fat-free mass, FM: fat mass, SM: skeletal muscle

* P<0.05, **P<0.01

Table 4-3. Multiple regression analysis with REE as the dependent variable and FFM, fat mass, plasma T_3, internal organs as the independent variables.

REE (kcal/day)	R^2	β	P value
Model 1	0.681		
FFM (kg)		0.825	<0.001
Model 2	0.683		
FFM (kg)		0.782	<0.001
Fat mass (kg)		0.067	0.597
Model 3	0.761		
FFM (kg)		0.697	<0.001
Fat mass (kg)		0.078	0.484
T_3 (ng/dL)		0.289	0.003
Model 4	0.786		
FFM (kg)		0.579	<0.001
Fat mass (kg)		0.002	0.989
T_3 (ng/dL)		0.283	0.002
Liver (kg)		0.236	0.068
Model 5	0.777		
FFM (kg)		0.627	<0.001
Fat mass (kg)		0.116	0.305
T_3 (ng/dL)		0.286	0.002
Brain (kg)		0.139	0.136
Model 6	0.761		
FFM (kg)		0.704	<0.001
Fat mass (kg)		0.082	0.479
T_3 (ng/dL)		0.290	0.003
Heart (kg)		-0.018	0.865
Model 7	0.769		
FFM (kg)		0.639	<0.001
Fat mass (kg)		0.038	0.749
T_3 (ng/dL)		0.256	0.010
Kidneys (kg)		0.135	0.305

REE: resting energy expenditure, FFM: fat-free mass,
T_3: plasma triiodothyronine

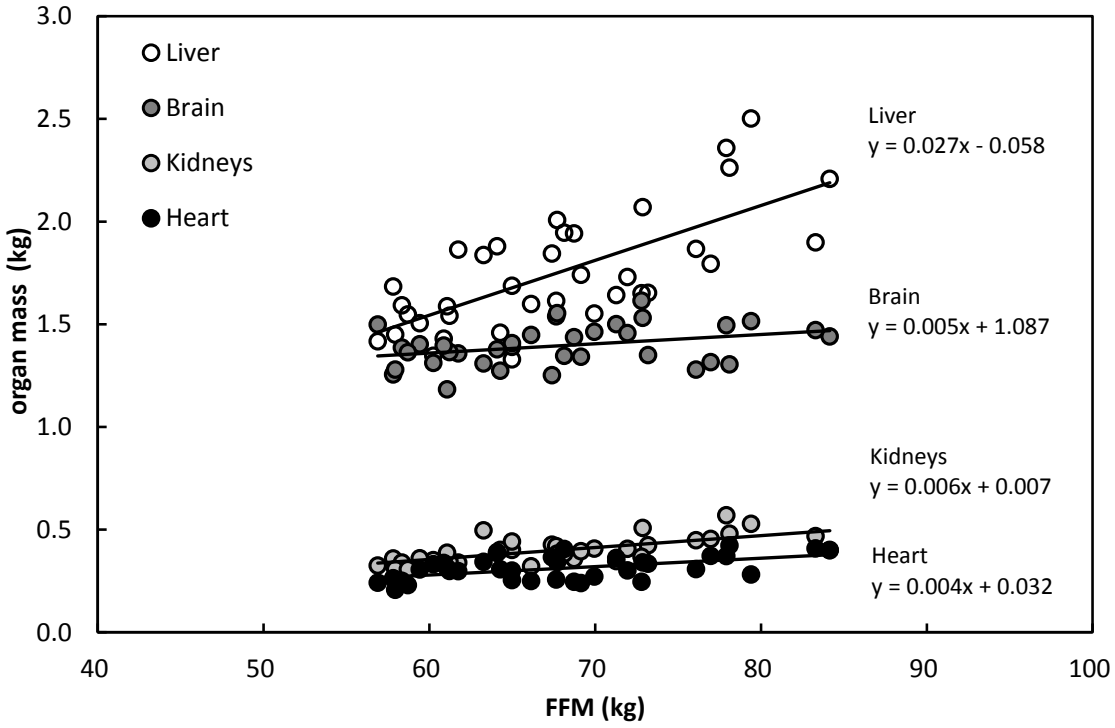

Figure 4-1 Relationship between fat-free mass and organ mass of liver, brain, kidneys and heart.

FFM: fat-free mass

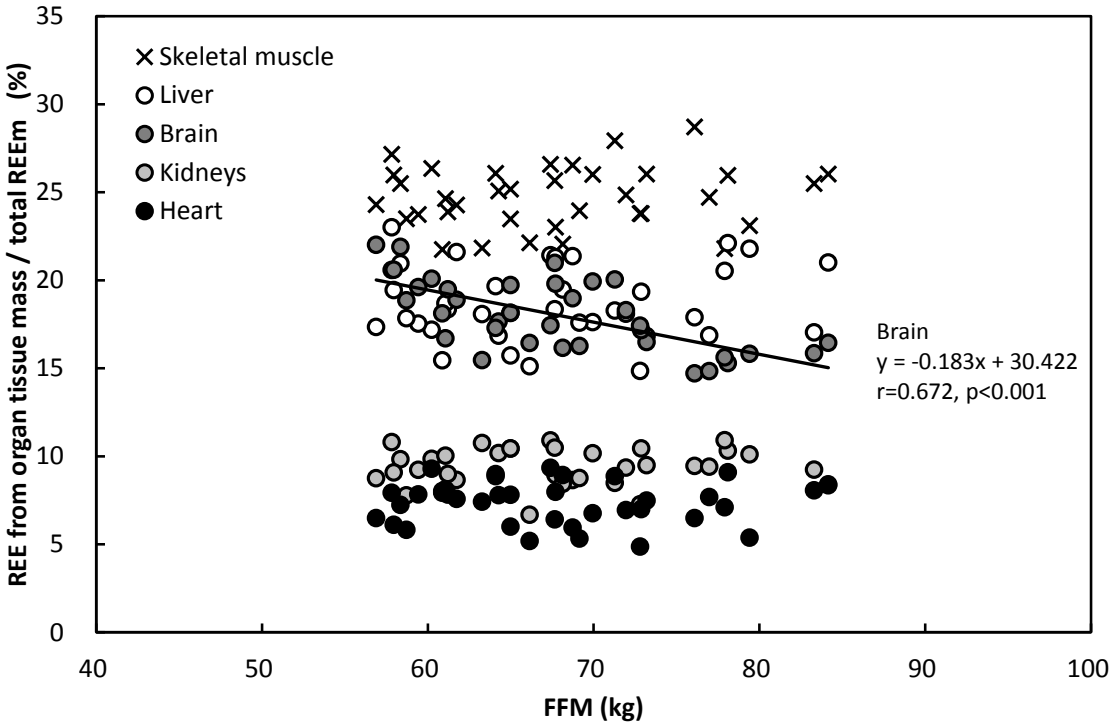

Figure 4-2 Relationship between fat-free mass and percentage contribution of resting energy expenditure from each tissue and organs mass to total measured REE.

FFM: fat-free mass, REEm: measured resting energy expenditure

REE of tissue and organs were calculated by each tissue and organs mass multiplied by the metabolic rate of each metabolic rates (skeletal muscle: 13 kcal/kg/day, liver: 200 kcal/kg/day, brain: 240 kcal/kg/day, kidneys: 440 kcal/kg/day, and heart: 440 kcal/kg/day)

Chapter 5

General discussion and conclusion

1) Background and purpose of the thesis

The energy balance management in sports is important in following points. Firstly, for all athletes, total energy intake must be raised to provide the energy expended during athletic training and performance. Secondly, in sports which small or large body weight is advantageous for performance, athletes practice weight gain or loss technique that place their metabolic and bone health as well as performance at risk. Previous research has identified high risk for metabolic syndrome and cardiovascular disease for large athletes such as American football players. Thirdly, during training and competition in sports of high intensity and long duration, the limiting factor for performance is energy intake. In order to meet individual daily energy requirement, REE become important parameter, since REE is basis for estimating total energy requirement.

Typically, FFM and REE/FFM ratio are recommended to utilize the estimation of REE for athletes. Many researchers have examined the relationship between body composition and REE based on the non-athletic healthy subjects. On the other hand, there are only a few researches have focused on the REE of athletic population who characterize in large muscle mass and regularly participate in high intensity physical activities. Therefore, it is unknown if the same findings based on non-athletic population would apply to athletic population. The purpose of the study was to investigate the relationship between body composition and REE of male athletes with special emphasis on FFM components including internal organs. By accomplishing the purpose, we expected to provide a basis for understanding REE of athletes and to contribute on the improvement of accurate and practical prediction of REE for athletes.

2) Summary of the studies

Study 1

The purpose was to evaluate the relationship between FFM and REE at the organ-tissue level for male athletes using a four organ-tissue compartment model. Fifty-seven healthy collegiate male athletes participated in this study. Measured REE showed significant correlation with FFM ($r=0.84$, $p<0.001$), on the other hand, REE/FFM ratio did not show any significant relationship with FFM (average REE/FFM: 27.8kcal/day). The relative contribution of bone mass, skeletal muscle, and residual mass were constant among the groups with different size of FFM. Therefore, it was suggested that the REE/FFM ratio is constant in male athletes in spite of the different FFM size, and it can be explained by the steadiness of relative contribution of FFM components to total FFM.

Study 2

The purpose was to investigate the influence of FFM gain on REE of male power athletes. Twenty-eight 1st year college American football players have participated in the longitudinal study for one year. The average weight gain was 7.4 ± 3.7kg with 9.8% increase after one year of weight gain period. REE/FFM ratio did not change regardless of 3.9 ± 2.1kg (6.0%) of FFM gain. The relative contribution of bone mass to REE remained unchanged after the 10% of weight gain, however, the skeletal muscle contribution increased by 0.8% and the residual mass contribution has decreased by 0.4%. Regardless of the changes in the relative contribution of skeletal muscle and residual mass to REE, since these changes were not large changes, REE/FFM ratio remained consistent after 3.9kg of FFM gain.

Study 3

The purpose was to examine the influence of internal organs mass to REE/FFM ratio of male power athletes. Subjects were thirty-seven college male American

football players. The liver, kidneys, and heart relative contribution to REE were consistent regardless of FFM, except for brain which was negatively correlated with FFM ($r=0.672$, $p<0.001$). FFM and T_3 (thyroid hormone) were the variance independently affected REE according to the multiple regression analysis. After FFM, FM and T_3 were adjusted, none of the organs were an independent factor which influences REE. Based on these results, the steady contribution of internal organs to REE other than brain is suggested to contribute on the consistency of REE/FFM ratio for male power athletes.

3) Conclusion and future development

Based on the results, the present studies suggested that FFM is the major determinant of REE for male athletes, and unlike the previous studies with non-athletic subjects, REE/FFM ratio was consistent regardless of FFM in our studies. The rational for the consistency of REE/FFM ratio in male athletes was due to the constant contribution of organs and tissues (bone, skeletal muscle, liver, heart and kidneys) on REE other than brain. The increase of adipose tissue in accordance with FFM may also have contributed to the maintenance of constant REE/FFM ratio of male power athletes, although it was probably a minor influence. Additionally, the REE/FFM ratio remained constant with 7.4kg of body weight gain including 3.9kg of FFM gain. However, the changes of relative contribution of skeletal muscle and residual mass suggested that there may be a decrease in REE/FFM ratio if athletes accomplished larger FFM gain. In addition to that, after the increase of body weight take place, the plasma level of T_3 which is related to a thermogenic adaptation became more influential for the variance of REE in male power athletes.

In this study we were able to find the relationship between FFM and REE at

organ-tissue level including individual organs for male athletes through cross-sectional and longitudinal studies. However, there are still other concerns regarding the REE of male athletes which need to be addressed. In the present study, the subjects were tended to be medium to large size male athletes (handball and American football players), therefore, it would be helpful to assess athletes from variety of sports types with relatively a smaller FFM in order to safely apply the findings from the present study to all types of athletes. As one of the other limitations of our study, the total increase of FFM was relatively small (3.9kg). As a consequence, it was difficult to observe the significant changes in FFM compositions and determine the clear influence of FFM on REE. The further investigation with greater FFM gain would be helpful to remove such issue and expect to clarify the influence of FFM components on REE. At last, even though we were able to find FFM components and T_3 as the independent factors, they were only able to explain up to 80% of the variance of REE for male athletes. In order to improve the accuracy of REE estimation, it is important to find other factors which determine the remaining 20% of variance for REE of male athletes.

4) Practical Application

As for the application to practical athletic scenes, the adequacy of estimating REE based on its individual FFM has been reassured among the male athletes with the ranged observed in the present studies (about 55kg to 85kg). However, it should not be completely disregarded about the risk on athletes with smaller or larger size of FFM which may result in under- or over-estimation of their REE.

When the athlete involves in significant weight gain (with 10% or more), the accuracy for predicting REE based on their FFM may be lowered. If there is a chance to measure a plasma level of thyroid hormone, perhaps it may help to

estimate individual REE more accurately for athletes who practice a weigh change.

The method to measure FFM may become a concern for some athletes with no access to the DXA or other elaborated machine to measure their body composition. Therefore, the FFM estimated by DXA and the FFM calculated based on the % body fat measured by the bioelectrical impedance analysis (Inner Scan BC-660, Tanita Co.) were compared, and found that there was a significant correlationship ($r=0.965$, $p<0.001$) between the two values. There should be a caution made for using bioelectric impedance analysis because it is influenced by a person's body water level or a body figure. Nonetheless, bioelectrical impedance analysis is possible to become a substitution for measuring FFM in the practical sports scenes.

It is ideal if individual REE can be measured by indirect calorimetry for athletes. Where it is difficult to measure REE directly, FFM can be used as a dependable factor to estimate REE which is the basis of predicting energy expenditure for athletes.

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